Pancreatic A-Cell Function in the Partially Pancreatectomized Otsuka Long-Evans Tokushima Fatty Rat, a Model of Spontaneous Non–Insulin-Dependent Diabetes Mellitus

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We examined whether a 70% pancreatectomy changes the morphofunctionality of pancreatic A cells in a model rat (Otsuka Long-Evans Tokushima Fatty [OLETF]) with non–insulin-dependent diabetes mellitus. Male OLETF rats aged 6 weeks were assigned to two groups: partial pancreatectomy (Px) and sham pancreatectomy (sham). The Px group was divided into three subgroups based on treatment received after surgery, which included treatment with nicotinamide, phlorhizin, or saline. As a control, their diabetes-resistant counterparts, Long-Evans Tokushima Otsuka (LETO) rats, were similarly treated and grouped. Six weeks after surgery, plasma glucagon responses to arginine- and insulin-induced hypoglycemia were examined. In addition, the glucagon content and morphological features of pancreatic A cells in Px-remnant and remnant-equivalent pancreata were investigated 7 weeks after surgery. A sustained nonfasting hyperglycemia was evident in Px OLETF rats, which was ameliorated by administration of nicotinamide. The glucagon content and A-cell mass were not decreased significantly in the remnant pancreas of saline- and phlorhizin-treated Px animals of either strain but increased in nicotinamide-treated animals compared with those in the remnant equivalent of the respective sham rats. The areas under the response curves of plasma glucagon (ΣIRG) during an arginine infusion test and 90 minutes of insulin-induced hypoglycemia were 1,010.7 ± 72.9, 1,083.1 ± 95.3, 1,029.6 ± 65.0, and 1,779.8 ± 226.9 pmol · L^{-1} · min^{-1} versus 1,997.0 ± 283.1, 2,217.0 ± 395.0, 1,479.6 ± 78.0, and 3,466.4 ± 174.0 pmol · L^{-1} · min^{-1} in phlorhizin-, nicotinamide-, and saline-treated Px OLETF and sham OLETF rats, respectively. A similar trend was observed for differences in the response of pancreatic A cells to both stimuli among various groups of LETO rats. There was no significant difference in ΣIRGs during both tests between OLETF and LETO rats with similar treatments, except during an insulin tolerance test (ITT) in saline-treated Px rats. The magnitude of the plasma glucagon response to both stimuli in the test animals was roughly parallel to the glucagon content in the pancreas. These findings suggest that differences in the proliferation and responsiveness of pancreatic A cells between OLETF and LETO rats after a 70% pancreatectomy are not nearly as significant as compared with B cells.

OTSUKA LONG-EVANS Tokushima Fatty (OLETF) rats, established by Kawano et al,[1] develop non–insulin-dependent diabetes mellitus at adulthood. In an earlier report, we showed that insulin resistance precedes the impairment of pancreatic B-cell function in this model rat,[2] and our previous study[3] showed that a 70% pancreatectomy resulted in a persistent elevation in nonfasting blood glucose level, which was associated with insufficient proliferation of B-cells in OLETF rats. In the same study, using diabetes-resistant Long-Evans Tokushima Otsuka (LETO) rats, B-cell mass increased sufficiently to suppress the elevation of nonfasting blood glucose levels after a 70% pancreatectomy. This suggests that the hyperglycemic OLETF rats are unable to respond to an increased insulin demand with a higher rate of B-cell compensatory growth. However, an important remaining issue is whether there are any alternations in A cells from the standpoint of the coordination of anatomically linked A cells and B cells[4] and the fact that the normal relationship between glucagon and insulin is impaired in all forms of diabetes.[4-7] This report describes a study of alternations in blood glucose levels after a partial pancreatectomy and A-cell morphofunctionality in the pancreatic remnant during either hyperglycemia or nondiabetic glycemia, induced by administration of phlorhizin, a renal glucose reabsorption inhibitor.[8] Data are also presented on the effects of administration of nicotinamide, a poly (ADP-ribose) synthetase inhibitor. Such an inhibitor could affect the response of remnant A cells to stimuli with amelioration of hyperglycemia, induced by increased growth of pancreatic B-cell mass.[9,10]

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Submitted November 19, 1995; accepted June 23, 1996.

Supported in part by a Grant-in-Aid for Scientific Research (07671142) from the Ministry of Education, Science, and Culture, Japan, and by a grant for a 5-year project on the Exploration of the Pathogenesis of Diabetes Mellitus from Otsuka Pharmaceutical.

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0026-0495/96/4511-0009$03.00/0

MATERIALS AND METHODS

Animals

A spontaneously diabetic rat with polyuria, polydipsia, and slight obesity was discovered in an outbred colony of Long-Evans rats that had been purchased from Charles River Canada (St. Constant, Quebec, Canada) in 1983 and subsequently maintained at the Tokushima Research Institute, Otsuka Pharmaceuticals (Tokushima, Japan). After 20 generations of selective breeding, a diabetic strain (OLETF) was established in 1990. According to Kawano et al,[1] the cumulative incidence of diabetes in male and female OLETF rats over 23 weeks of age is 86.0% and 0%, respectively. OLETF and LETO male rats aged 5 weeks were obtained from the Tokushima Research Institute and maintained in our animal facilities under specific pathogen-free conditions (Institute of Animal Experimentation, University of Tokushima). The temperature (21 ± 2°C), humidity (55% ± 5%), lighting (7:00 AM to 7:00 PM), and air conditioning were controlled. The animals were supplied with standard rat chow (Oriental Yeast, Tokyo, Japan) and tap water ad libitum.

Experimental Design

After acclimatization for 1 week, experiments were performed on rats at 6 weeks of age at the beginning of the study. The rats

were allocated at random into two groups: partial pancreatectomy (Px) and sham pancreatectomy (sham). The Px group was divided into three subgroups based on treatment received after surgery, which included treatment with nicotinamide, phlorhizin, or saline. A total of 12 rats were used for each subgroup. LETO rats were used as controls.

After overnight fasting, animals were anesthetized with ether and given additional ether, if needed, during surgery. All pancreatic tissue was removed by gentle abrasion with cotton applicators, except for an anatomically well-defined remnant bordered by the branch of the hepatic portal vein and the first portion of the duodenal loop. The sham operation was performed by disengaging the pancreas from the mesentery and gently rubbing it between the fingers. Following surgery, rats received food and water ad libitum.

During the first week after surgery, body weight and nonfasting blood glucose concentration were measured daily at 4:00 to 5:00 PM, and thereafter once per week at the same time of day. Blood samples were obtained by tail snipping.

Phlorhizin (400 mg/kg body weight/d), prepared as a 20% solution in propylene glycol, was administered subcutaneously in three equal doses at 8-hour intervals to ensure continuous inhibition of renal tubular glucose reabsorption.[8] Nicotinamide (350 mg/kg body weight/d) or normal saline was injected intraperitoneally once per day. The injections were initiated 3 days after surgery, when hyperglycemia first became evident in Px OLETF rats, and continued until the end of the experiments. The last injections were given 15 hours before killing.

Glucagon Secretion and Content

An arginine test (AT) was performed 6 weeks after surgery. After an overnight fast, rats were anesthetized as described earlier. An arginine solution (10% L-arginine-HCl) was infused at a rate of 20 mg/kg body weight/min for 30 minutes through a catheter inserted into the femoral vein, and 0.4-mL blood samples were collected before and 15, 30, and 45 minutes after the arginine infusion to determine blood glucose and glucagon levels.

An insulin tolerance test (ITT) was performed 6 weeks after surgery. After an overnight fast, rats were anesthetized as already described. After obtaining a basal blood sample, insulin was injected as an intravenous (IV) bolus at a dose of 0.7 to 1.5 U/kg body weight, depending on the blood glucose level. Samples of blood were withdrawn before and 30, 45, 60, and 90 minutes after the injection to determine blood glucose and plasma glucagon levels.

To avoid heavy stress on the animals during this limited treatment period, half of the rats in each group were subjected to the AT and the remaining rats to the ITT.

After 1 week's recovery from the AT or ITT (7 weeks after surgery), the rats were fasted and treated with sodium pentobarbital (60 mg/kg body weight), and the abdomen was quickly opened and the pancreas removed. Each intact pancreas or Px remnant was excised, cleared of lymph nodes and fat, and weighed. A portion of each tissue with the same anatomic orientation was cassetted, placed in Bouin's fixative for 4 hours, rinsed in running water for 24 hours, fixed in 10% buffered Formalin, and then embedded in paraffin using a standard protocol. The remaining tissues were stored at −80°C. In a later experiment, all samples were individually homogenized using a polytron homogenizer (Kinematica, Luzern, Switzerland) with 6 mL cold acid-ethanol (750 mL absolute alcohol, 250 mL distilled water, and 15 mL concentrated HCl) per gram of tissue, kept at 4°C overnight, and centrifuged at 600 × *g* for 30 minutes. The supernatant was stored at −80°C until assayed for glucagon.

Quantitative Morphometrics

Two sets of three serial sections (3 to 5 μm thick) were obtained at intervals of approximately 250 μm. The sections were first deparaffinized, and then immunostained with an ABC kit (Amersham, Amersham, UK) for glucagon immunostaining. The primary antibody used was polyclonal rabbit antiglucagon antibody (1:1,000; Dako, Carpinteria, CA). Using Weibel's point-counting morphometrics,[11] the relative volumes of both islet and A-cell masses (percent) were quantified at 200× magnification on a monitor screen using an Olympus microscope connected to a color video camera and an image analysis system (Tokyo, Japan). Starting at a random point in one corner of the section, the islet mass and A-cell mass were scored in every other field using a 96-point grid with a minimum of 4,800 points in 50 fields counted per tissue block. The relative volumes of both A-cell and islet masses were calculated by dividing the counts of intersections over the A-cell mass or islet mass by the total counts of intersections over the pancreatic tissue. The intercepts over blood vessels, fat, ducts, or interlobular space were subtracted to obtain total pancreatic counts. The absolute A-cell mass was then calculated for each rat by multiplying the relative volume by the pancreatic weight (the results are expressed in micrograms). All observations were made by one person (M.Z.).

Assays

Blood glucose values were determined by the glucose oxidase method. Radioimmunoassay of glucagon immunoreactivity (IRG) was performed with antiserum OAL-123 (Otsuka Pharmaceutical, Tokushima, Japan), which is directed toward the free C-terminal end of glucagon and recognizes glucagon but not glicentin or oxyntomodulin.[12]

Statistical Analysis

The data are reported as the mean ± SEM unless otherwise indicated. Significance was determined by ANOVA, followed by Tukey's test of individual comparison of means.

RESULTS

Nonfasting Blood Glucose Concentration and Body Weight

Nonfasting blood glucose levels in saline-treated Px OLETF rats began to increase on the first day after surgery, and remained above 16.7 mmol/L (300 mg/dL) after the fourth day ($P < .01$ *v* all other groups; Table 1). In Px OLETF rats, these values sharply decreased following phlorhizin injection and subsequently remained near the sham levels. However, nonfasting blood glucose values for nicotinamide-treated Px OLETF rats decreased gradually, and remained at levels that were significantly higher than in sham rats but significantly lower than in saline-treated rats, and approached near-sham levels 28 days after surgery. In contrast to Px OLETF rats, no hyperglycemia was observed in Px LETO rats. Nonfasting blood glucose values for all LETO rats were maintained within a nondiabetic range from 5.0 to 7.0 mmol/L (90 to 126 mg/dL) throughout the experiments, although at several time points nonfasting blood glucose levels in Px LETO rats were significantly higher than in the sham counterparts. The increase in body weight was significantly slower in saline-treated Px OLETF rats than in phlorhizin- and nicotinamide-treated and sham OLETF rats during the observation period. No apparent

Table 1. Effect of 70% Px on Nonfasting Blood Glucose Levels

Days After Surgery	Nonfasting Blood Glucose (mmol/L)							
	OLETF				LETO			
	Px				Px			
	Phlorhizin	Nicotinamide	Saline	Sham	Phlorhizin	Nicotinamide	Saline	Sham
0	6.9 ± 0.2	6.8 ± 0.4	6.8 ± 0.1	6.8 ± 0.1	6.4 ± 0.2	6.1 ± 0.1	6.0 ± 0.2	6.2 ± 0.2
1	7.9 ± 0.3‖	8.6 ± 0.3‖	10.3 ± 1.9	6.8 ± 0.1	6.8 ± 0.2‖	6.3 ± 0.1‖	6.5 ± 0.4	5.6 ± 0.1
2	9.6 ± 0.6‖	9.4 ± 1.1§	12.7 ± 1.9‖	6.5 ± 0.1	6.4 ± 0.2§	6.2 ± 0.3	6.9 ± 0.3‖	5.7 ± 0.2
3	6.8 ± 0.4	11.7 ± 1.1‡	12.9 ± 1.7‡	6.4 ± 0.2	6.5 ± 0.3§	6.6 ± 0.2‖	7.0 ± 0.3‖	5.6 ± 0.2
4	7.7 ± 0.4	9.9 ± 1.3‡	24.2 ± 0.5†	6.9 ± 0.2	6.2 ± 0.2	6.6 ± 0.4	6.7 ± 0.3	5.8 ± 0.1
5	6.8 ± 0.3	9.0 ± 1.2‡	18.6 ± 0.2†	6.3 ± 0.2	6.6 ± 0.4‖	6.6 ± 0.2‖	6.4 ± 0.4§	5.2 ± 0.1
6	7.6 ± 0.4‖	8.6 ± 0.8‡	19.9 ± 0.8†	6.0 ± 0.2	7.0 ± 0.3‖	6.7 ± 0.3§	6.2 ± 0.2	5.7 ± 0.2
7	6.3 ± 0.5	9.1 ± 1.2‡	18.8 ± 0.6†	5.8 ± 0.3	6.0 ± 0.3	6.6 ± 0.2	6.6 ± 0.4§	5.4 ± 0.2
14	6.0 ± 0.3	8.0 ± 1.7‡	19.0 ± 0.8†	5.7 ± 0.2	6.9 ± 0.5	6.4 ± 0.3	6.3 ± 0.1	5.2 ± 0.1†
21	6.2 ± 0.1	7.9 ± 0.5‡	22.0 ± 1.1†	5.6 ± 0.3	5.6 ± 0.2	6.3 ± 0.3‖	6.2 ± 0.2‖	5.1 ± 0.1
28	6.9 ± 0.3	7.4 ± 0.4§	24.6 ± 2.5†	6.2 ± 0.3	6.0 ± 0.2	6.4 ± 0.1	6.7 ± 0.1	5.0 ± 0.1†
35	7.9 ± 0.3‖	7.6 ± 0.7	16.8 ± 2.5†	6.1 ± 0.1	5.9 ± 0.2	5.7 ± 0.2	6.0 ± 0.1‖	5.5 ± 0.1

NOTE. Data are the mean ± SEM from 12 rats for each condition. Phlorhizin 400 mg/kg body weight/d and nicotinamide 350 mg/kg body weight/d were injected daily beginning the third day after surgery and continuing until the day of killing. The last injections were given 15 hours before death.

*$P < .05$, †$P < .01$: *v* all other groups for each strain.

‡$P < .01$ *v* both phlorhizin and sham groups.

§$P < .05$, ‖$P < .01$: *v* sham group.

retardation of the increase in body weight was found in Px LETO rats compared with sham rats (Table 2).

Tissue Weight, Glucagon Content, and A-Cell Mass

Px remnant and remnant-equivalent pancreatic weights were expressed as a percentage of the total pancreatic weight from the sham counterparts, adjusted for body weight (at the time of killing). The results showed that remnant-equivalent pancreata from sham rats in both strains remained at approximately 30% of total pancreatic weight and contained approximately 30% of the glucagon content. The Px remnant from saline-treated Px LETO rats increased in weight, reaching 38.2% ± 3.1% of the total pancreatic weight, while the remnant from the OLETF counterparts constituted 27.4% ± 3.3% (Fig 1). The percent weights of the Px remnant in phlorhizin- and nicotinamide-treated Px OLETF rats were significantly lower than in the Px LETO counterparts (Fig 1). However, the glucagon content in the Px remnant from the OLETF group was higher than the value predicted from the tissue weight. In nicotinamide-treated Px rats of both strains (Table 3), it is clear that a considerable increase in A-cell mass occurred (1.6-fold increase in Px OLETF rats and 2.8-fold increase in Px LETO rats *v* saline-treated rats) and that this increased growth of A-cell mass was consistent with an increase in glucagon content. No significant differences in glucagon content and A-cell mass in the remnant pancreata from phlorhizin- and saline-treated Px OLETF rats were observed, suggesting that hyperglycemia per se has no significant effect on these parameters.

Plasma IRG Response to Arginine and Insulin-Induced Hypoglycemia

Blood glucose levels during the AT in nicotinamide-treated Px OLETF rats were intermediate between those obtained in saline-treated Px OLETF and sham OLETF rats, and were significantly different from each other at several time points (Fig 2). The same trend was observed for LETO groups, although the difference was not as dramatic as for the OLETF groups. Plasma IRG levels increased significantly from the basal level of 12.6 ± 0.6 pmol/L in sham OLETF rats and 11.6 ± 0.3 pmol/L in

Table 2. Effect of 70% Px on Body Weight

Days After Surgery	Body Weight (g)							
	OLETF				LETO			
	Px				Px			
	Phlorhizin	Nicotinamide	Saline	Sham	Phlorhizin	Nicotinamide	Saline	Sham
0	205.2 ± 4.1	193.0 ± 4.3	187.6 ± 3.9	181.3 ± 6.5	163.9 ± 2.4	165.2 ± 2.6	165.2 ± 2.6	168.7 ± 2.7
7	229.5 ± 3.9	230.6 ± 5.0	219.3 ± 4.6*	232.3 ± 7.5	182.4 ± 4.2	188.4 ± 2.6	193.0 ± 1.4	210.2 ± 2.5
14	278.4 ± 4.0‡	270.2 ± 3.7‡	252.1 ± 5.8*	277.3 ± 8.2	230.0 ± 4.5	240.0 ± 2.8	230.3 ± 3.5	240.8 ± 3.2
21	318.4 ± 4.0‡	304.1 ± 2.9	291.0 ± 5.8†	323.3 ± 8.0	260.3 ± 5.0*	270.0 ± 2.9	272.8 ± 3.3	275.5 ± 4.0
28	330.0 ± 3.6	329.0 ± 12.0	310.0 ± 10.6*	339.6 ± 5.0	284.0 ± 5.5	282.2 ± 3.2	303.7 ± 2.3	299.5 ± 4.3
35	356.0 ± 3.2	350.0 ± 13.8	326.0 ± 19.0	361.0 ± 4.4	298.3 ± 7.8	283.5 ± 7.0*	303.6 ± 3.4	305.0 ± 3.1

NOTE. Data are the mean ± SEM from 12 rats for each condition.

*$P < .05$, †$P < .01$: *v* sham group.

‡$P < .05$ *v* saline group.

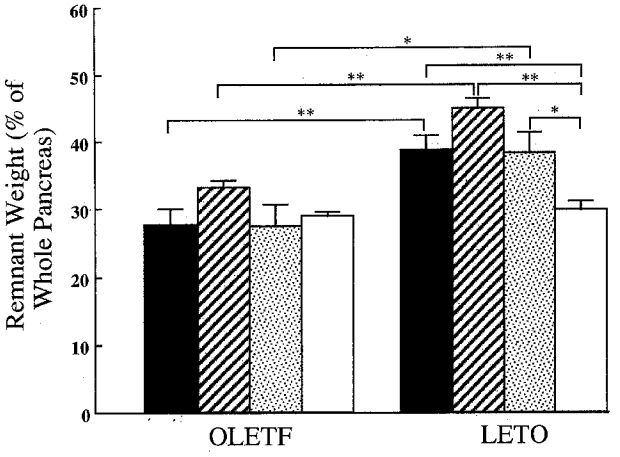

Fig 1. Increased growth of the remnant pancreas in Px + phlorhzin (■), Px + nicotinamide (▨), Px + saline (▩), and sham (□) rats of both strains 7 weeks after surgery. Values are the percentage of the remnant weight relative to the whole pancreatic weight from the counterpart sham rats, mean ± SEM from 12 rats for each condition. *$P < .05$, **$P < .01$.

sham LETO rats, reaching peaks of 81.6 ± 14.3 pmol/L in the former and 55.0 ± 3.9 pmol/L in the latter at 30 minutes. Px OLETF rats, irrespective of the treatment, showed a similarly attenuated plasma IRG response to an arginine load as compared with sham OLETF rats, peak values for the former being 27 to 29 pmol/L, approximately 30% of the value for the latter. The same trend, despite not being as dramatic as for OLETF rats, was observed in Px LETO rats, except for the nicotinamide-treated group. The total areas of the IRG responses (ΣIRGs) to arginine stimulation were 39.2% to 43.3% less in Px OLETF rats than in sham OLETF rats. The difference in ΣIRGs during the AT between Px and sham LETO rats was not as remarkable as that between Px and sham OLETF rats.

Blood glucose and plasma IRG responses to IV insulin injection in OLETF and LETO rats are shown in Fig 3. Blood glucose levels were not significantly different among the various groups after IV injection of insulin. Blood glucose levels decreased, reaching nadirs of less than 2.0 mmol/L at 45 or 60 minutes for all groups, although the levels in OLETF rats were slightly higher than those in LETO rats. However, there was no significant difference between the levels in the various groups of the same strain. Plasma IRG responses to insulin-induced hypoglycemia were similar to those in both sham OLETF and LETO rats (12.7 ± 0.9 to 73.1 ± 9.6 pmol/L *v* 14.3 ± 4.6 to 62.4 ± 3.1 pmol/L). Plasma IRG increased slightly but significantly from the basal level of 15.2 ± 1.4 pmol/L to a peak of 32.8 ± 9.3 pmol/L in nicotinamide-treated Px OLETF rats. In contrast, there was no significant increase in plasma IRG levels after IV injection of insulin in saline- and phlorhizin-treated Px OLETF rats. However, in Px LETO rats, a significant increase in plasma IRG during insulin-induced hypoglycemia was observed to various extents among the groups. The difference in ΣIRGs during the ITT between Px and sham LETO rats was not as dramatic as that between Px and sham OLETF rats. The percent change in ΣIRG during the ITT in one Px group in comparison to the sham group was roughly similar to the value during the AT in the corresponding groups.

There were no significant differences in ΣIRGs during both tests between OLETF and LETO rats with similar treatments, except during the ITT in saline-treated Px rats (Table 4).

DISCUSSION

In OLETF rats at 4 weeks after a 70% pancreatectomy,[3] B-cell mass in the remnant pancreas decreased to 19.4% of the total B-cell mass, while that in the remnant equivalent was 29.1%, close to the expected value of 30%. This

Table 3. Glucagon Content and A-Cell Mass of the Remnant From Px Rats and the Remnant Equivalent of Whole Pancreas From Sham Rats

Animals (n)	Glucagon Content (ng)	Glucagon Concentration (ng/g)	Relative A-Cell Mass	A-Cell Mass	A-Cell Mass/ Islet Mass	Percentage of Total A-Cell Mass
OLETF						
Px						
Phlorhizin (6)	41.9 ± 9.1	191.8 ± 49.24	0.2 ± 0.03	408.0 ± 79.6	10.4 ± 2.9	17.2 ± 3.2‡
Nicotinamide (6)	49.1 ± 5.9	206.4 ± 23.6	0.2 ± 0.03	589.6 ± 105.2	12.4 ± 0.3‡	24.8 ± 4.4§
Saline (6)	40.0 ± 5.0‡	209.1 ± 22.2§	0.2 ± 0.1	372.8 ± 145.1	12.8 ± 3.4	15.7 ± 6.1
Sham (6)						
Remnant equivalent	32.8 ± 6.6	142.0 ± 25.4	0.2 ± 0.04	432.3 ± 104.3	14.0 ± 1.9	19.5 ± 5.2
Whole pancreas	94.1 ± 5.1		0.4 ± 0.1	2,377.2 ± 321.6	18.7 ± 1.9	
LETO						
Px						
Phlorhizin (6)	32.3 ± 5.2*	84.8 ± 14.1	0.1 ± 0.03	441.3 ± 96.9†	12.1 ± 2.6*	29.6 ± 2.7†
Nicotinamide (6)	56.6 ± 8.3	146.7 ± 21.9	0.2 ± 0.02	896.5 ± 99.1	19.9 ± 1.9	50.1 ± 5.5
Saline (6)	20.2 ± 4.2†	57.6 ± 13.0	0.1 ± 0.02	320.2 ± 91.2†	10.8 ± 2.9*	17.9 ± 5.1†
Sham (6)						
Remnant equivalent	28.1 ± 11.7†	110.1 ± 48.5	0.2 ± 0.04	388.7 ± 122.7†	13.4 ± 3.6	23.7 ± 6.9†
Whole pancreas	89.4 ± 28.5		0.3 ± 0.1	1,787.8 ± 282.6	20.1 ± 1.8	

NOTE. Data are the mean ± SEM.

*$P < .05$, †$P < .01$: *v* nicotinamide rats.

‡$P < .05$, §$P < .01$: *v* LETO counterparts.

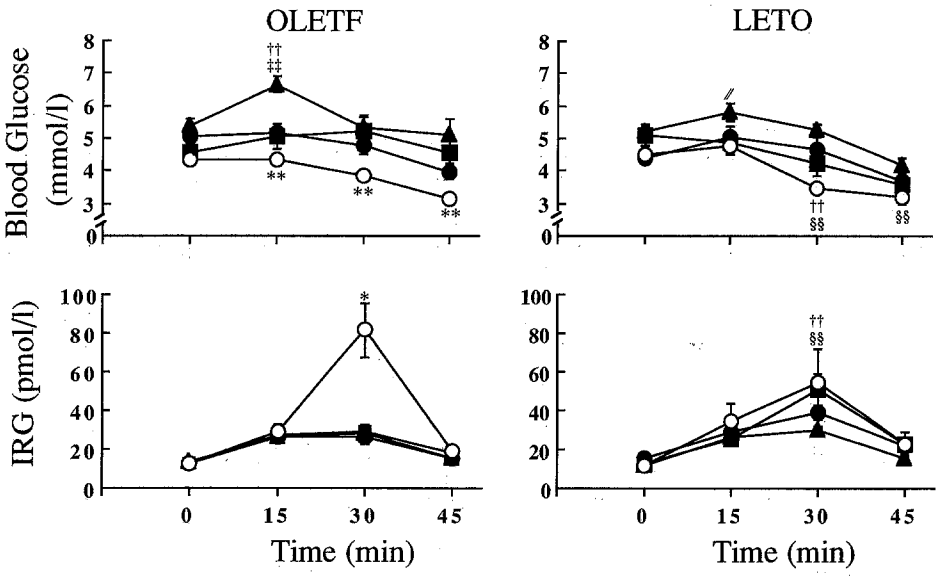

Fig 2. Blood glucose and plasma IRG responses to IV infusion of arginine in Px + phlorhizin (●), Px + nicotinamide (■), Px + saline (▲), and sham (○) rats of both strains 6 weeks after surgery. (The animals were used for the data in Table 3.) Points and bars represent the mean ± SEM. *$P < .05$, **$P < 0.01$: *v* all other groups. ††$P < .01$ *v* Px + phlorhizin. ‡‡$P < .01$ *v* Px + nicotinamide. §§$P < .01$ *v* Px + saline. //$P < .01$ *v* sham.

decrease in B-cell mass in the remnant pancreas was probably due to an inherent low capacity for B-cell proliferation as a result of the stress of the partial Px and the subsequent inevitable changes resulting from structural exhaustion of pancreatic B cells. It is known that hyperglycemia leads to premature death of B cells and their subsequent replacement by fibrous tissue.[13] The present study clearly shows that A-cell mass and glucagon content in the remnant pancreas of Px OLETF rats were not significantly decreased compared with those in the remnant equivalent. In addition, it is clear that nicotinamide treatment leads to an increase in A-cell mass and glucagon content in remnant pancreata from both Px OLETF and Px LETO rats, which is associated with increased plasma glucagon responses to arginine and insulin-induced hypoglycemia. The percent total A-cell mass in remnant-equivalent pancreata from sham OLETF and LETO rats was 19.5% ± 5.2% and 23.7% ± 6.9%, respectively. These values were less than predicted from the percentage of whole pancreatic weight in the remnant equivalent, assuming that A cells may distribute equally throughout the pancreas. The remnant pancreas, which represents the middle portion of the pancreatic head in this study, was reported to be poor in glucagon-containing cells and rich in pancreatic polypeptide–containing cells.[14] This may explain why the percent A-cell mass was lower than the percent pancreatic weight of the remnant-equivalent pancreas. Furthermore, in OLETF rats, the difference in A-cell mass between remnant pancreata from saline-treated Px rats and remnant-equivalent pancreata from sham rats (15.7% *v* 19.5%) was not as remarkable as that for B-cell mass between saline-treated Px rats and their sham counterparts (19.4% *v* 29.1%, $P < .01$).[3] The fact that glucagon content in remnant and remnant-equivalent pancreas was approximately 30% of

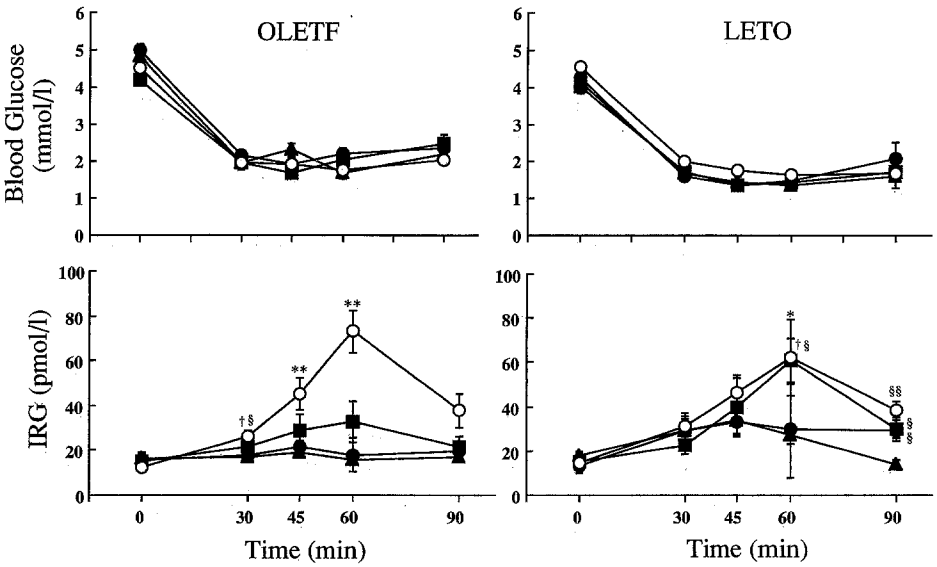

Fig 3. Blood glucose and plasma IRG responses to IV injection of insulin in Px + phlorhizin (●), Px + nicotinamide (■), Px + saline (▲), and sham (○) rats of both strains 6 weeks after surgery. Points and bars represent the mean ± SEM. (The animals were used for the data in Table 3.) *$P < .05$, **$P < .01$ *v* all other groups. †$P < .05$ *v* Px + phlorizin. §$P < .05$, §§$P < .01$ *v* Px + saline.

Table 4. ΣIRG After IV Arginine and Insulin in OLETF and LETO Rats

Group	No. of Rats		ΣIRG (pmol · L^{-1} · min^{-1})	
	AT	ITT	AT	ITT
OLETF				
Px				
Phlorhizin	6	5	1,010.7 ± 72.9*	1,997.0 ± 283.1†
Nicotinamide	6	5	1,083.1 ± 95.3*	2,217.0 ± 395.0*
Saline	6	5	1,029.6 ± 65.0*	1,479.6 ± 78.0†‡
Sham	6	5	1,779.8 ± 226.9	3,466.4 ± 174.0
LETO				
Px				
Phlorhizin	6	5	1,193.3 ± 182.3*	2,456.1 ± 180.7*
Nicotinamide	6	5	1,420.0 ± 346.3	3,144.8 ± 310.9
Saline	6	5	1,044.6 ± 73.4†	2,248.7 ± 236.8*
Sham	6	5	1,602.9 ± 128.1	3,559.3 ± 364.7

NOTE. Data are the mean ± SEM. Administration routes and doses of arginine and insulin were the same as for Figs 2 and 3, respectively.

*$P < .05$, †$P < .01$: *v* sham rats.

‡$P < .05$ *v* LETO counterparts.

the total glucagon content suggests that individual A cells located in these portions of the pancreas contain more glucagon than the other regions.

Nicotinamide prevented an increase in the nonfasting blood glucose level caused by a 70% Px in OLETF rats. The partial Px removed a substantial mass of glucagon-containing A cells, and this loss was expected to ameliorate hyperglycemia. However, this is not the case in Px OLETF rats treated with nicotinamide, since this treatment increased the glucagon content and A-cell mass in the Px remnant. Taken together with the results of previous reports, the findings suggest that nicotinamide prevents the development of hyperglycemia in partial Px animals by stimulating B-cell regeneration.[15-17] There has been no report that nicotinamide stimulates the proliferation of A cells, but it has been reported that it induces glucagon and insulin mRNA in cultured human fetal islets.[17] This effect may be in part responsible for the increased glucagon content in the remnant pancreas from Px animals treated with nicotinamide.

To our knowledge, this is the first report showing that nicotinamide treatment increases A-cell regeneration in remnant pancreata from both Px OLETF and LETO rats. It seems reasonable to presume that nicotinamide acts on the putative pluripotent islet stem cell and stimulates its differentiation.[18] We were able to identify numerous newly formed islets that contained only A cells, present near the ducts, which may be one of the reasons for the observed increased ratio of A-cell mass to islet mass. This finding suggests that nicotinamide triggers a process in the uncommitted epithelial cells, which eventually results in expression of the islet cell phenotype. The pancreatic A-cell mass and glucagon content in nicotinamide-treated Px OLETF rats tended to be higher than in the sham counterparts, but the difference in these parameters in both groups was not significant. However, when the A-cell mass in the remnant pancreas was calculated as a percentage of total A-cell mass in the whole pancreas, there was clearly a 5.3% increased growth of A-cell mass in the remnant pancreas from nicotinamide-treated Px OLETF rats with a 17.3% increase in glucagon content as compared with the remnant-equivalent pancreas from the sham rats. This unparalleled increase in A-cell mass and glucagon content in Px rats treated with nicotinamide may be a reflection of a higher quantity of glucagon stored or synthesized per unit of A-cell mass.

There was a marked difference in nonfasting blood glucose levels of phlorhizin- and saline-treated Px OLETF rats, the maximum value being 7.9 mmol/L during the treatment in the former and 24.6 mmol/L during the observation period in the latter (Table 1). There were no significant differences in glucagon content and A-cell mass in the remnant pancreata or in plasma glucagon response to arginine and glucopenia in both groups. We used phlorhizin here to avoid the possible impairment effects of hyperglycemia on A-cell function and proliferation. As expected, the hyperglycemia was normalized with phlorhizin. These findings suggest that hyperglycemia per se is not a sufficient condition to lead to a deleterious effect on pancreatic A cells, in contrast to its toxic effect on pancreatic B cells.[19-21] It should be noted that in phlorhizin-treated Px OLETF rats, blood glucose levels at two time points after injection were slightly but significantly higher than those in the sham counterparts. A possible reason is that an interval of 8 hours to the next injection was too long for continuous inhibition of renal tubular glucose reabsorption. However, this difference seems unlikely to result in toxic effects on pancreatic islet cells.

Basal blood glucose levels in saline-treated Px OLETF rats were not significantly higher than in sham OLETF rats, despite the marked elevation in nonfasting glucose (Figs 2 and 3). The same observation was reported in a previous study,[22] ie, blood glucose levels remained normal during the fasting state, but during hyperglycemia, the levels approached 17 to 20 mmol/L in OLETF rats aged 16 and 24 weeks, after an oral glucose load. Whether this disproportionate postprandial hyperglycemia is a particular feature of this rat model is unknown. It is possible that the basal insulin level was high enough to control the fasting blood glucose level, while the plasma insulin response to glucose was not high enough to suppress postprandial glucose in this diabetic state. Fasting hyperglycemia becomes evident at age 65 weeks in OLETF rats, when the plasma immunoreactive insulin level decreases markedly.[1]

It has been reported that A-cell responsiveness to glucopenia was impaired but the responsiveness to arginine was maintained in OLETF rats aged 16 and 24 weeks, and that an impaired glucagon response to glucopenia was not observed in the younger rats.[23] This lack of an A-cell response to hypoglycemia in older OLETF rats has been speculated to be due to an altered morphological relationship between A and B cells, rather than an alteration in A-cell function. Sham OLETF rats showed a distinct increase in plasma glucagon in response to glucopenia in the present study. The test was performed in the rats at age 12 weeks, when the A cells were intact in terms of function

and morphology. The diminished IRG response to insulin in saline-treated Px OLETF rats might not be an appropriate adaptation to the preexisting chronic nonfasting hyperglycemia, because a decreased IRG response to insulin was also found in phlorizin-treated Px OLETF rats. It has been reported that A-cell responsiveness to hypoglycemia was correlated with insulin secretory ability, but the responsiveness to arginine was different.[24,25] The responsiveness of pancreatic A cells as evaluated by the plasma glucagon response to arginine- and insulin-induced hypoglycemia in Px rats appears to be roughly parallel to the glucagon content in the remnant pancreata. Remnant pancreata from both the nicotinamide-treated Px OLETF and Px LETO rats contained 52.0% and 62.9% of the total glucagon content in the whole pancreata of the corresponding sham rats, respectively. ΣIRGs during the AT and ITT in nicotinamide-treated Px OLETF rats were 60.9% and 64.0% of the values in sham OLETF rats. ΣIRGs during both tests in nicotinamide-treated Px LETO rats were 88.5% and 88.4% of the values in sham LETO rats. In saline-treated Px OLETF rats, the plasma glucagon response to glucopenia was indiscernible, whereas plasma glucagon levels increased distinctly, even though the response to arginine infusion was slight. In contrast, in LETO rats with similar treatment, plasma glucagon responses to glucopenia were evident. This discrepancy between pancreatic A-cell responses to arginine and glucopenia may be due to a dramatic decrease in insulin content in the remnant pancreata from saline-treated Px OLETF rats, with the same being true for the difference in plasma IRG response to glucopenia between saline-treated Px OLETF and LETO rats (insulin content, 4.4 ± 1.3 μg in remnant pancreas of saline-treated Px OLETF *v* 19.2 ± 5.1 μg in saline-treated Px LETO, $P < .01$). However, the differences in the insulin content of remnant pancreata from both Px OLETF and LETO rats with phlorhizin and nicotinamide treatments were not as remarkable as those in both saline-treated Px OLETF and Px LETO rats (10.6 ± 2.2 and 16.5 ± 2.8 μg in phlorizin- and nicotinamide-treated Px OLETF, respectively *v* 17.4 ± 5.2 and 22.8 ± 3.2 μg in Px LETO treated similarly). This may be one of the reasons there was no significant difference in ΣIRG during the ITT between OLETF and LETO rats with similar treatment, except in saline-treated Px rats.

The percentage values for the remnant pancreatic weights were lower in Px OLETF rats with the various treatments than in the Px LETO counterparts (Fig 1). This is largely due to poor proliferation of the pancreatic exocrine tissues and B cells after a partial Px in OLETF rats.[3] Large differences in the capacity for proliferation of pancreatic A cells after partial Px and the response to stimuli between OLETF and LETO rats would not be expected. The percentage of total A-cell mass was significantly lower in phlorhizin-treated and nicotinamide-treated Px OLETF rats than in the LETO counterparts (Table 3). If the capacity for proliferation of pancreatic A cells were impaired in OLETF rats, the A-cell mass would be lower in OLETF rats than in LETO rats; however, this is not the case. Based on these data, we cannot say unequivocally that pancreatic A cells of OLETF rats have a poorer capacity for proliferation after partial Px compared with those of LETO rats.

We conclude from this study that a 70% Px induced an elevation of nonfasting blood glucose in OLETF rats, which was ameliorated by administration of nicotinamide. The glucagon content and A-cell mass not decreased significantly in remnant pancreata of saline- and phlorhizin-treated rats, but were increased in nicotinamide-treated Px animals of both strains, as compared with those in the remnant equivalent of the respective sham rats. The magnitude of the plasma glucagon response to arginine and hypoglycemia was lower in Px versus sham rats, and was to a certain extent in parallel with the glucagon content. These findings suggest that the differences in proliferation and responsiveness of pancreatic A cells between OLETF and LETO rats after a 70% Px are not nearly as significant as those of pancreatic B cells.

REFERENCES

1. Kawano K, Hirashima T, Mori S, et al: Spontaneous long-term hyperglycemic rat with diabetic complications, Otsuka Long-Evans Tokushima Fatty (OLETF) strain. Diabetes 41:1422-1428, 1992

2. Ishida K, Mizuno A, Zhu M, et al: Which is the primary etiological event in Otsuka Long-Evans Tokushima Fatty rats, a model of spontaneous non–insulin-dependent diabetes mellitus, insulin resistance, or impaired insulin secretion? Metabolism 44:940-945, 1995

3. Zhu M, Noma Y, Mizuno A, et al: Poor capacity for proliferation of pancreatic B-cells in Otsuka-Long-Evans-Tokushima fatty rat. Diabetes 45:941-946, 1996

4. Weir GC, Bonner-Weir S: Islets of Langerhans: The puzzle of intraislet interactions and their relevance to diabetes. J Clin Invest 85:983-987, 1990

5. Unger RH, Aguiar-Parada E, Muller WA, et al: Studies of pancreatic alpha cell function in normal and diabetic subjects. J Clin Invest 49:837-848, 1970

6. Muller WA, Faloona GR, Aguiar-Parada E, et al: Abnormal alpha-cell function in diabetes. Response to carbohydrate and protein ingestion. N Engl J Med 283:109-115, 1970

7. Muller WA, Faloona GR, Unger RH: The effect of experimental insulin deficiency on glucagon secretion. J Clin Invest 50:1992-1999, 1971

8. Rossetti L, Smith D, Schulman GI, et al: Correction of hyperglycemia with phlorizin normalizes tissue sensitivity to insulin in diabetic rats. J Clin Invest 79:1510-1515, 1987

9. Yonemura Y, Takashima T, Miwa K, et al: Amelioration of diabetes mellitus in partially depancreatized rats by poly (ADP-ribose) synthetase inhibitors. Diabetes 33:401-403, 1984

10. Sugiyama K, Yonemwa Y, Okamoto H: Effects of poly (ADP-ribose) synthetase inhibitor on B-cells of a canine pancreas after massive pancreatectomy. Int J Pancreatol 8:85-95, 1992

11. Weibel ER: Principles and methods for the morphometric studies of the lung and other organs. Lab Invest 12:131-155, 1963

12. Nishino T, Kodaira T, Shin S, et al: Glucagon radioimmunoassay with use of antiserum to glucagon C-terminal fragment. Clin Chem 27:1690-1697, 1981

13. Legg MA, Harawi SJ: The pathology of diabetes mellitus, in

Marble AL, Krall P, Bradley RF, et al (eds): Joslin's Diabetes Mellitus. Philadelphia, PA, Lea & Febiger, 1985, pp 298-307

14. Malaisse-Lagae F, Stephan Y, Cox J, et al: Identification of a lobe in the adult human pancreas rich in pancreatic polypeptide. Diabetologia 17:361-365, 1979

15. Sandler S, Anderson A: Long-term effects of exposure of pancreatic islets to nicotinamide in vitro on DNA synthesis, metabolism and B-cell function. Diabetologia 29:199-202, 1986

16. Sandler S, Anderson A, Korsgren O, et al: Tissue culture of human fetal pancreas. Effects of nicotinamide on insulin production and formation of islet-like cell clusters. Diabetes 38:168-171, 1989 (suppl 1)

17. Otonkoshi T, Beattie GM, Mally MI, et al: Nicotinamide is a potent inducer of endocrine differentiation in cultured human fetal pancreatic cells. J Clin Invest 92:1459-1466, 1993

18. Alpert S, Hanahan D, Teitelman G: Hybrid insulin genes reveal a developmental lineage for pancreatic endocrine cells and imply a relationship with neurons. Cell 53:295-308, 1988

19. Bonner-Weir S, Trent DF, Weir GC: Partial pancreatectomy in the rat and subsequent defect in glucose-induced insulin release. J Clin Invest 71:1544-1553, 1983

20. Robertson RP, Zhang HJ, Pyzdrowski KL, et al: Preservation of insulin mRNA levels and insulin secretion in HIT cells by avoidance of chronic exposure to high glucose concentrations. J Clin Invest 90:320-325, 1992

21. Olson LK, Redmon JB, Towle HC, et al: Chronic exposure of HIT cells to high glucose concentrations paradoxically decreases insulin gene transcription and alters binding of insulin gene regulatory protein. J Clin Invest 92:514-519, 1993

22. Shima K, Shi K, Sano T, et al: Is exercise training effective in preventing diabetes mellitus in the Otsuka-Long-Evans-Tokushima Fatty rat, a model of spontaneous non–insulin-dependent diabetes mellitus? Metabolism 42:971-977, 1993

23. Ishida K, Mizuno A, Sano T, et al: Plasma glucagon responses to insulin-induced hypoglycemia and arginine in spontaneous non–insulin-dependent diabetes mellitus (NIDDM) rats, Otsuka Long Evans Tokushima Fatty (OLETF) strain. Acta Endocrinol (Copenh) 129:585-593, 1993

24. Shima K, Tanaka R, Sawazaki N, et al: Relationship between secretory capacity of pancreatic A cell and B cell. Horm Metab Res 11:451-452, 1979

25. Fukuda M, Tanaka A, Tahara Y, et al: Correlation between minimal secretory capacity of pancreatic B-cells and stability of diabetic control. Diabetes 37:81-88, 1988